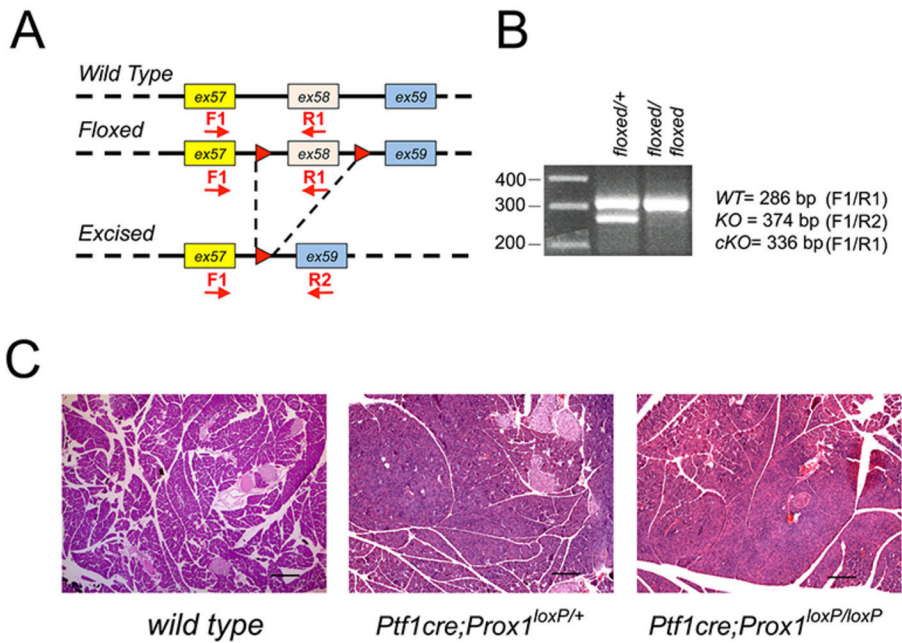
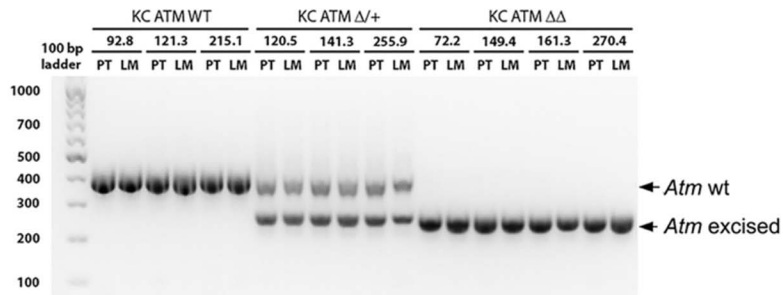


**ATM-deficiency increases genomic instability and metastatic potential in a mouse model
of pancreatic cancer**

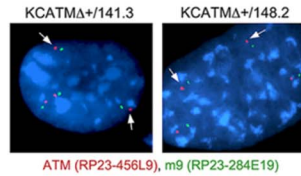
Yiannis Drosos, David Escobar, Ming-Yi Chiang, Kathryn Roys, Virginia Valentine, Marc B. Valentine, Jerold E. Rehg, Vaibhav Sahai, Lesa A. Begley, Jianming Ye, Leena Paul, Peter J. McKinnon, Beatriz Sosa-Pineda.



Supplementary Figure 1. Conditional deletion of *Atm* in the pancreas. **A:** Schematic representation of the *Atm* locus in *wildtype*, *Atm^{LoxP}* (Floxed allele), and *Ptf1a-Cre;Atm^{LoxP}* (Excised allele). Exons 57, 58 and 59 in the *Atm* coding (kinase domain) region are shown with colored boxes. Under the activity of Cre recombinase exon 58 is excised, resulting in a kinase-deficient ATM protein. **B:** Primers F1 and R1 amplify a 286 bp product from the *wildtype* allele and a 336 bp product from the *LoxP*-containing allele ("Floxed"). Primers F1 and R2 amplify a 374 bp sequence from the *excised* allele. **C:** H&E staining shows that pancreas histology is comparable amongst *wildtype*, *Ptf1-Cre;Atm^{LoxP/+}* (*Atm* pancreas heterozygous) and *Ptf1-Cre;Atm^{LoxP/LoxP}* (*Atm* pancreas-homozygous) adult mice. Scale bars: 100µm.



		Norm. expr.(10 ⁻⁵)	SD (10 ⁻⁵)
KC	92.8	1.4	9.6
	121.3	0.2	2.8
	215.1	0.7	4.8
KCAΔ+	120.5	43.1	4.4
	141.3	49.1	4.2
	255.9	119.0	42.6
KCAΔΔ	72.2	61.9	0.5
	149.4	32.6	0.1
	161.3	46.7	0.4



ATM (RP23-456L9), m9 (RP23-284E19)

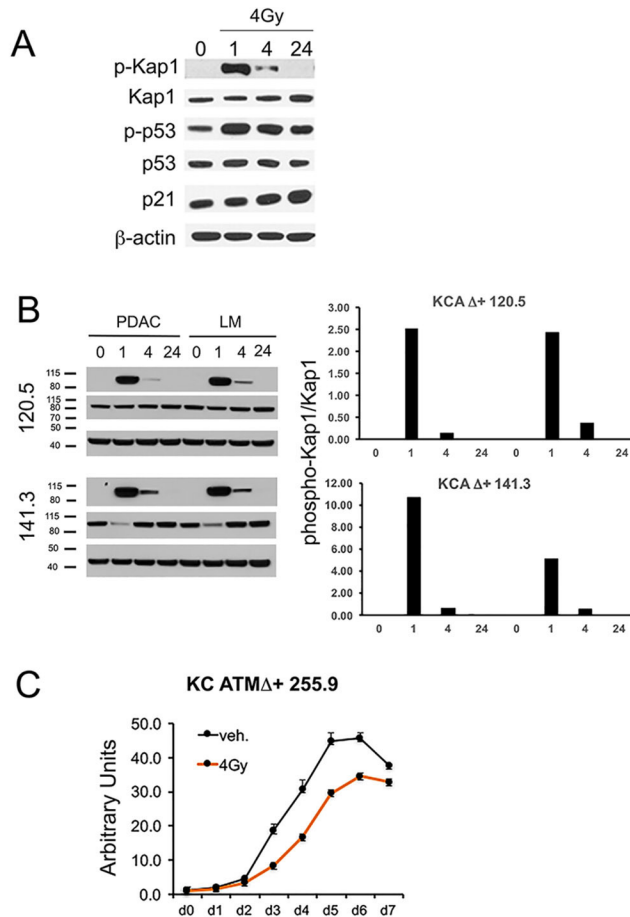
Supplementary Figure 2. *KCATMΔ+* tumor cell lines do not show Loss of Heterozygosity (LOH).

A: RT-PCR results using cDNA isolated from *KC*, *KCATMΔ+* and *KCATMΔΔ* primary tumor cell lines and primers spanning exon 58 (Forward: TGTGGTTCTGATGGCAAGGAAAG. Reverse: CAAACCAAACAGCTGGGTCCAAGA). PCR products of the expected sizes from both the *Atm* wildtype allele and the *Atm* (exon 58) floxed allele are amplified from all the *KCATMΔ+* samples.

B: qPCR results showing the abundance of a fragment spanning exon 58 relative to that of beta-actin in PDAC cell lines of the 3 genotypes.

C: FISH analysis shows no evidence of large deletions in the *Atm* locus in 2 independent primary *KCATMΔ+* tumor cell lines (141.3 and 148.2).

The red probe hybridizes to a region in chromosome 9 where *Atm* is located; the green probe hybridizes to a region in chromosome 9 outside the *Atm* locus.



Supplementary Figure 3. *KCATM* Δ + tumor cells show activation of signals downstream of ATM upon irradiation. **A:** Western blots show induction of phospho-Kap1, phospho-p53 and p21 in MEFs upon 4Gy IR exposure. **B:** (*left*) Western blots showing phospho-Kap1 induction 1-4hr post-irradiation in *KCATM* Δ + 120.5 and 141.3 tumor cells. (*right*) Quantification of Western blot results by densitometry. **C:** *KCATM* Δ + 255.9 (PDAC) cells do not display significant radiosensitization in an MTT assay. (Error bars represent \pm SEM values of triplicate experiments, 2-Way ANOVA.)

Supplementary Table 1. Survival group tumor analysis

	ID	Genotype	Age (days/months)	Pancreatic tumor	Metastasis			Ascites	Other		
					Liver	Diaphragm	Lung				
	1	KCATMΔΔ	140/5 (E)	Y	Y	Y	Y	Y	Peritoneum		
	2		154/5 (E)	Y	Y	Y	N	Y			
	3		80.6	83/6 (E)	Y	Y	NA	NA		NA	
	4		120.4	200/7 (E)	Y	Y	N	N		Y	
	5		270.4	222/7 (FD)	Y	Y	N	N		NA	
	6		162.3	199/7 (FD)	Y	Y	N	N		NA	
	7		161.3	239/8 (E)	Y	Y	N	N		Y	
	8		270.4	222/8 (E)	Y	Y	N	N		N	
	9		111.4	239/8 (FD)	Y	Y	N	N		NA	
	10		72.2	263/9 (E)	Y	N	N	N		N	
	11		280.4	275/9 (FD)	Y	Y	N	N		NA	
	12		72.4	252/10 (E)	Y	N		N		Y	
	13		164.4	281/10 (FD)	N	N	N	N		NA	
	14		155.6	276/10 (FD)	Y	Y	N	N		NA	
	15		72.6	293/10 (FD)	Y	Y	N	N		NA	
	16		162.4	318/11 (E)	Y	Y	N	N		Y	Obstructed bile duct, jaundice
	17		73.7	348/12 (E)	Y	Y	N	N		Y	
	18		98.4	361/12 (E)	Y	Y	N	N		Y	Liver cirrhosis
	1	KCATMΔ+	90/3.5 (E)	N	N	N	N	N	Extensive inflammation		
	2		111.8	150/5 (E)	N	N	N	N	N	Hind limb paralysis, ataxia, spinal tumor	
	3		115.1	180/6 (E)	N	N	N	N	N	Hypoglycemia	
	4		141.3	229/8 (E)	Y	Y	N	N	Y		
	5		148.2	223/8 (E)	Y	Y	N	N	Y		
	6		111.1	240/8 (FD)	Y	Y	N	N	NA		
	7		255.9	254/9 (E)	Y	Y	N	N?	Y	Cysts	
	8		167.9	274/9 (FD)	Y	Y	N	N	NA		
	9		148.4	275/9 (FD)	Y	Y	N	N	NA		
	10		120.7	273/9 (FD)	N	N	N	N	NA		
	11		172.6	291/10 (FD)	N	N	N	N	NA		
	12		133.1	331/11 (FD)	Y	Y	N	N	NA		
	13		161.8	324/11 (FD)	Y	Y	N	N	NA		
	14		167.8	321/11 (FD)	N	N	N	N	NA		
	15		167.6	364/12 (FD)	N	N	N	N	NA	Cysts	
	16		120.3	424/14 (FD)	Y	Y	N	N	NA		
	17		121.4	404/14 (FD)	Y	Y	N	N	NA		
	18		120.5	469/16 (E)	Y	Y	Y	N	Y		
	19		165.1	474/16 (E)	Y	Y	Y	Y	Y		
	20		121.1	517/17 (E)	N	N	N	N		Cysts	
	21		193.7	516/17 (E)	Y	Y	N	Y	Y		
	1	KC	201/7 (E)	Y	Y	N	N	Y			
	2		215.1	207/7 (E)	Y	Y	N	N	Y		
	3		88.8	274/10 (E)	N	N	N	N	N		
	4		309.1	294/10 (FD)	Y	N	N	N	NA		
	5		14226 40.2	304/10 (FD)	N	N	N	N	NA		
	6		14226 36.1	330/11 (FD)	Y	N	N	N	NA		
	7		14226 36.3	375/12 (FD)	N	N	N	N	NA		
	8		121.3	375/13 (E)	Y	Y	N	N	Y	Liver cirrhosis	
	9		72.5	384/13 (E)	N	N	N	N	N	Cysts	
	10		285.8	409/14 (E)	N	N	N	N	N		

11	147.1	466/15 (FD)	N	N	N	N	NA
12	92.4	451/15 (FD)	N	N	N	N	NA
13	80.1	498/16 (FD)	Y	Y	N	N	NA
14	97.5	492/16 (FD)	N	N	N	N	NA
15	14226 36.2	527/17 (FD)	N	N	N	N	NA
16	13430 97.8	528/18 (FD)	N	N	N	N	NA
17	27.6	508/19 (FD)	Y	N	N	N	NA
18	97.9	610/21 (E)	Y	Y	Y	N	Y
19	132.8	718/24 (FD)	N	N	N	N	NA

E: Euthanized

FD: Found dead

NA: Not Applicable

Supplementary Table 2. QPCR results showing CT values normalized to β -actin.

			PROGENITOR										SQUAMOUS				IMMUNOGENIC				ADEX			
			<i>Lgals4</i>		<i>Hnf1B</i>		<i>Gata6</i>		<i>Pdx1</i>		<i>HNF4a</i>		<i>Egfr</i>		<i>Prrx1</i>		<i>Igll1</i>		<i>Igj</i>		<i>Rbpjl</i>		<i>Cpa2</i>	
			CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD	CT ave	CT STD
KC	92.8	PT	4.11.	1.07.	6.40.	1.67.	1.41.	3.91.	2.83.	7.38.	1.93.	6.73.	8.91.	2.40.	7.01.	1.49.	2.20.	5.82.	6.49.	1.85.	0.00.	0.00.	0.00.	0.00.
		LM	E-05	E-05	E-04	E-04	E-02	E-03	E-03	E-04	E-07	E-08	E-04	E-04	E-03	E-03	E-07	E-08	E-07	E-07	E+00	E+00	E+00	E+00
	121.3	PT	3.38.	8.20.	2.01.	4.86.	7.63.	1.84.	6.34.	1.53.	8.99.	3.99.	9.05.	2.18.	8.90.	5.13.	2.09.	5.74.	5.99.	2.20.	8.67.	8.64.	0.00.	0.00.
		LM	E-05	E-06	E-03	E-04	E-02	E-02	E-03	E-03	E-07	E-07	E-04	E-04	E-03	E-04	E-07	E-08	E-07	E-07	E-08	E-08	E+00	E+00
	215.1	PT	1.93.	2.81.	4.40.	6.11.	6.72.	9.72.	1.04.	1.47.	1.38.	2.17.	4.48.	6.23.	3.03.	1.46.	1.80.	3.10.	2.09.	3.48.	1.91.	1.36.	0.00.	0.00.
		LM	E-02	E-03	E-03	E-04	E-01	E-02	E-02	E-03	E-05	E-06	E-04	E-05	E-06	E-07	E-07	E-08	E-07	E-08	E-08	E-08	E+00	E+00
KC ATM $\Delta/+$	120.5	PT	3.28.	1.05.	1.45.	1.66.	1.17.	1.34.	2.51.	2.87.	4.51.	5.25.	8.70.	9.96.	1.36.	1.32.	1.51.	2.00.	9.52.	1.29.	0.00.	0.00.	0.00.	0.00.
		LM	E-02	E-02	E-02	E-03	E+00	E-01	E-02	E-03	E-05	E-06	E-04	E-05	E-06	E-06	E-07	E-07	E-07	E-07	E+00	E+00	E+00	E+00
	141.3	PT	7.45.	1.75.	1.61.	2.72.	4.77.	8.04.	3.67.	6.20.	3.45.	7.30.	1.49.	2.50.	6.81.	3.93.	1.28.	3.23.	4.63.	9.33.	0.00.	0.00.	0.00.	0.00.
		LM	E-04	E-04	E-03	E-04	E-02	E-03	E-03	E-04	E-06	E-07	E-04	E-05	E-05	E-06	E-07	E-08	E-08	E-09	E+00	E+00	E+00	E+00
	255.9	PT	4.36.	8.69.	1.22.	1.08.	7.84.	9.41.	2.41.	2.18.	5.75.	5.14.	1.01.	9.90.	2.85.	1.07.	3.94.	3.94.	4.90.	1.25.	0.00.	0.00.	0.00.	0.00.
		LM	E-03	E-04	E-02	E-03	E-01	E-02	E-02	E-03	E-05	E-06	E-03	E-05	E-04	E-05	E-06	E-07	E-07	E-07	E+00	E+00	E+00	E+00
KC ATM Δ/Δ	72.2	PT	1.53.	9.64.	1.14.	1.63.	1.15.	2.89.	1.65.	1.50.	7.34.	1.01.	2.67.	1.63.	8.88.	1.16.	1.31.	1.06.	3.88.	1.13.	0.00.	0.00.	0.00.	0.00.
		LM	E-02	E-04	E-02	E-04	E+00	E-02	E-02	E-04	E-05	E-06	E-03	E-04	E-06	E-06	E-06	E-07	E-07	E-07	E+00	E+00	E+00	E+00
	141.3	PT	2.71.	1.11.	5.48.	2.85.	6.34.	2.60.	1.36.	5.56.	4.55.	1.82.	8.38.	3.33.	2.11.	1.92.	4.58.	8.33.	2.47.	5.76.	8.14.	4.48.	1.63.	3.04.
		LM	E-02	E-03	E-03	E-04	E-01	E-02	E-02	E-04	E-05	E-06	E-04	E-05	E-05	E-06	E-07	E-08	E-07	E-08	E-08	E-08	E-07	E-08
	255.9	PT	3.40.	4.61.	1.06.	1.06.	7.96.	2.54.	2.07.	2.79.	6.00.	5.82.	1.83.	3.87.	5.41.	2.37.	8.12.	3.37.	6.13.	3.31.	3.18.	9.59.	0.00.	0.00.
		LM	E-03	E-04	E-02	E-04	E-01	E-02	E-02	E-04	E-05	E-06	E-03	E-05	E-05	E-06	E-07	E-08	E-07	E-08	E-07	E-08	E+00	E+00
KC ATM $\Delta\Delta$	72.2	PT	6.70.	2.17.	6.64.	1.41.	2.37.	5.06.	1.13.	2.41.	3.54.	7.60.	9.58.	2.10.	4.96.	4.55.	2.71.	5.89.	5.33.	1.53.	1.34.	2.86.	4.40.	1.18.
		LM	E-04	E-04	E-03	E-03	E-01	E-02	E-02	E-03	E-05	E-06	E-04	E-04	E-05	E-06	E-07	E-08	E-07	E-07	E-07	E-08	E-08	E-08
	149.4	PT	3.99.	3.71.	4.82.	4.91.	6.87.	5.96.	3.46.	2.93.	5.13.	6.07.	1.34.	1.30.	5.10.	1.03.	1.03.	1.58.	1.62.	3.89.	1.82.	3.45.	0.00.	0.00.
		LM	E-04	E-05	E-02	E-03	E-01	E-02	E-02	E-03	E-05	E-06	E-03	E-04	E-05	E-06	E-06	E-07	E-06	E-07	E-07	E-08	E+00	E+00
	161.3	PT	7.44.	3.68.	5.26.	5.81.	3.91.	3.89.	1.85.	2.03.	1.32.	5.02.	4.40.	2.30.	5.95.	3.85.	7.62.	1.73.	3.70.	1.40.	2.28.	7.92.	0.00.	0.00.
		LM	E-03	E-04	E-02	E-04	E-01	E-03	E-02	E-04	E-05	E-07	E-04	E-05	E-06	E-07	E-07	E-08	E-07	E-07	E-07	E-08	E+00	E+00
270.4	PT	2.83.	1.38.	1.01.	4.56.	2.20.	2.12.	1.15.	5.19.	9.03.	1.31.	1.87.	8.57.	1.71.	1.56.	2.80.	1.60.	1.19.	3.05.	2.49.	1.27.	0.00.	0.00.	
	LM	E-02	E-03	E-02	E-04	E+00	E-01	E-02	E-04	E-05	E-05	E-03	E-05	E-04	E-05	E-06	E-07	E-06	E-07	E-07	E-08	E+00	E+00	
161.3	PT	9.71.	1.08.	1.04.	1.12.	7.67.	8.22.	1.62.	1.69.	4.05.	4.22.	9.35.	9.75.	1.09.	1.41.	1.74.	1.85.	6.70.	8.89.	1.15.	4.61.	1.12.	4.40.	
	LM	E-03	E-03	E-02	E-03	E-01	E-02	E-02	E-03	E-05	E-06	E-04	E-05	E-04	E-05	E-06	E-07	E-07	E-08	E-07	E-08	E-07	E-08	
270.4	PT	4.48.	2.34.	2.96.	1.25.	2.85.	1.43.	7.73.	3.07.	1.35.	5.67.	2.38.	1.55.	8.14.	3.29.	6.21.	3.53.	2.42.	3.97.	0.00.	0.00.	0.00.	0.00.	
	LM	E-03	E-04	E-03	E-04	E-01	E-02	E-03	E-04	E-05	E-07	E-04	E-05	E-06	E-07	E-07	E-08	E-07	E-08	E+00	E+00	E+00	E+00	
270.4	PT	8.25.	2.08.	4.51.	1.07.	1.72.	4.07.	7.88.	1.88.	9.68.	2.32.	3.09.	7.34.	6.81.	9.52.	3.47.	9.23.	2.06.	9.40.	0.00.	0.00.	0.00.	0.00.	
	LM	E-03	E-03	E-03	E-03	E-01	E-02	E-03	E-03	E-06	E-06	E-04	E-05	E-06	E-07	E-07	E-08	E-07	E-08	E+00	E+00	E+00	E+00	
270.4	PT	6.66.	1.30.	6.12.	1.15.	1.40.	2.63.	2.04.	3.95.	3.36.	8.41.	1.10.	2.06.	4.53.	2.07.	5.87.	1.96.	5.75.	1.53.	3.94.	4.35.	1.33.	2.65.	
	LM	E-02	E-02	E-03	E-03	E+00	E-01	E-02	E-03	E-05	E-06	E-03	E-04	E-05	E-06	E-07	E-07	E-07	E-07	E-08	E-08	E-07	E-08	
270.4	PT	1.26.	3.26.	2.68.	5.55.	8.69.	1.82.	2.27.	4.75.	7.29.	1.76.	7.84.	1.63.	3.03.	1.50.	1.94.	6.48.	8.09.	2.23.	0.00.	0.00.	0.00.	0.00.	
	LM	E-01	E-02	E-02	E-03	E-01	E-01	E-02	E-03	E-05	E-05	E-04	E-04	E-04	E-06	E-06	E-07	E-07	E-07	E+00	E+00	E+00	E+00	
270.4	PT	3.88.	2.43.	5.89.	2.73.	1.95.	1.33.	6.61.	2.65.	1.73.	2.03.	2.78.	1.11.	2.25.	2.65.	3.64.	5.19.	1.97.	3.58.	2.19.	3.71.	6.52.	3.16.	
	LM	E-03	E-04	E-03	E-04	E-01	E-02	E-03	E-04	E-05	E-06	E-04	E-05	E-05	E-06	E-07	E-08	E-07	E-08	E-08	E-09	E-08	E-08	
270.4	PT	3.92.	6.85.	7.01.	1.22.	1.82.	3.17.	7.13.	1.23.	9.63.	1.68.	2.62.	4.57.	6.77.	1.92.	1.92.	3.32.	1.83.	4.48.	0.00.	0.00.	0.00.	0.00.	
	LM	E-03	E-04	E-03	E-03	E-01	E-02	E-03	E-03	E-06	E-06	E-04	E-05	E-05	E-06	E-07	E-08	E-07	E-08	E+00	E+00	E+00	E+00	
PCRS.																				8.90.	3.58.	6.74.	7.38.	
																					E-03	E-04	E-01	E-03

Supplementary Table 3. Primary and secondary antibodies used in the study

Primary antibodies					
Antigen	Species	Source	Catalogue number	Paraffin (P) Frozen (F) Cultured cells (C) Western blot (WB)	Dilution
β-actin	Mouse	Sigma	A2228	WB	1:100,000
TROMA-III/CK19*	Rat	DSHB, U. of Iowa		P	1:100
F4/80	Rat	ABD Serotech	MCA497GA	1:1,000	P
γH2AX	Rabbit	Cell Signaling	9718	P F, C	1:500 1:100
KAP1	Rabbit	Bethyl	A300-274A	P, F, C WB	1:1,000 1:2,000
Phospho-KAP1	Rabbit	Bethyl	A300-767A	P, F, C WB	1:100 1:250
Ki67	Rabbit	Thermo	RM-9106	P, F, C	1:500
p16Ink4a	Rabbit	Santa Cruz	SC-1207	P, C	1:100
p19/Arf	Rat	LifeSpan	LS-C2744	P, C	1:500
p21	Mouse	Santa Cruz	SC-6246	P WB	1:100 1:500
p53	Rabbit	Proteintech	10442-1-AP	WB	1:1,000
Phospho-p53	Rabbit	Cell Signaling	9284	WB	1:250
Hnf1β	Rabbit	ProtechGroup	12533-1-AP	P	1:5,000
Secondary antibodies					
Antigen	Species	Source	Catalogue number	Dilution	Conjugate
Rabbit IgG	Donkey	Jackson Lab	711-065-152	1:500	Biotinylated
Rat IgG	Goat	Jackson Lab	112-065-003	1:500	Biotinylated
Sheep IgG	Donkey	Jackson Lab	713-065-003	1:500	Biotinylated
Rabbit IgG	Donkey	Jackson Lab	711-165-152	1:500	Cyanine Cy-3
Rabbit IgG	Donkey	Molecular Probes	A21206	1:500	Alexa-488
Rat IgG	Donkey	Molecular Probes	A21208	1:500	Alexa-488
Sheep IgG	Donkey	Jackson Lab	713-165-003	1:500	Cyanine Cy-3

*The TROMA-III antibody developed by R. Kemler was obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242.

Supplementary Table 4. qRT-PCR primers used for tumor classification

Gene		5' – 3'
<i>Igj</i>	Forward Primer	TGACGACGAAGCGACCATTC
	Reverse Primer	TTCAAAGGGACAACAATTCGGA
<i>Igll1</i>	Forward Primer	ACTCTGTTCCCTGCCTTCCTTA
	Reverse Primer	GAGGGTTGGGTTGTCTCTACA
<i>Cpa2</i>	Forward Primer	AAGAGCACCTTGAGCTTGATTT
	Reverse Primer	GAACTCGGACATGGACTGTCT
<i>Rbpjl</i>	Forward Primer	ACCGTCTGCGGTTACATGG
	Reverse Primer	GGTCTTGGCACAACCAAATTC
<i>Egfr</i>	Forward Primer	GCCATCTGGGCCAAAGATACC
	Reverse Primer	GTCTTCGCATGAATAGGCCAAT
<i>Lgals4</i>	Forward Primer	GGTCGTGGTGAACGAAATTC
	Reverse Primer	GTGGAGGGTTGTACCCAGGA
<i>Prrx1</i>	Forward Primer	AGCAGACGAAAGTGTGGGC
	Reverse Primer	TCAGAGTTCAACTGGTCATTGTC
<i>Trp53</i>	Forward Primer	CTTACCAGGGCAACTATGGCTT
	Reverse Primer	GCCAGCTGGCAGAATAGCTTATTG
<i>p19 Arf</i>	Forward Primer	GCTCTGGCTTTCGTGAACATGTTG
	Reverse Primer	TTCGAATCTGCACCGTAGTTGAGC
<i>p16 Ink4a</i>	Forward Primer	AACTCTTTCGGTCGTACCCCGATT
	Reverse Primer	ATCTGCACCGTAGTTGAGCAGAAG
<i>Gata6</i>	Forward Primer	GCTCTGTCCCTATGACTCCTACTT
	Reverse Primer	TTCTCCCACTGCAGACATCACT
<i>Hnf4a</i>	Forward Primer	CCTTATGCCCTCACAGGGTC
	Reverse Primer	GACATCCTCCTCCTGGTCCT
<i>Hnf1b</i>	Forward Primer	AGGGAGGTGGTCGATGTCA
	Reverse Primer	TCTGGACTGTCTGGTTGAACT
<i>Pdx1</i>	Forward Primer	AGCTCCCTTTCCCGTGGATGAAAT
	Reverse Primer	AGGCAGTACGGGTCCTCTTGTTTT
<i>B-actin</i>	Forward Primer	AGATCAAGATCATTGCTCCTCCT
	Reverse Primer	ACGCAGCTCAGTAACAGTCC

Supplementary Table 5. RT-PCR primers used for sequencing

Description	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Trp53</i> Exons 2-3 (set1)	ACCCTTTCCTATAAGCCATAG	GTCCATGCAGTGAGGTGATGG
<i>Trp53</i> Exons 2-3 (set2)	ATGACTGCCATGGAGGAGTCA	CAGGATATCTTCTGGAGGAAG
<i>Trp53</i> Ex. 4	TGGTAAGGCCCCAGAGCAGAA	GAGATGCAGAGAATATGAGA
<i>Trp53</i> Ex. 5	AATGGTGCTTGGACAATGTGTTTCA	ACCCGGATAAGATGCTGGGGA
<i>Trp53</i> Ex. 6	CGATGGTGATGGTAAGCCCTCAA	TAGCTAGCACTCAGGAGGGT
<i>Trp53</i> Ex. 7	AGAGGCTATAGCCAGCCATT	GGAACCAAAGAGCGTTGGGCAT
<i>Trp53</i> Ex. 8	TCTTATCTGTGGCTTCTCGG	TTGTGCAGGTGGGCAGCGCTGTGG
<i>Trp53</i> Ex. 9	GAAGTCCTTTGCCCTGAACT	TTTTATACATGCGAGAGACA
<i>Trp53</i> Ex. 10	ACCTGTAAGTGGAGCCAGCT	AGCAGGGGTGGGGTTTTTATC
<i>Trp53</i> Ex. 11	CCAGCCTAGAGCCTTCCAAGCCTTGA	TTGTGTCTCAGCCCTGAAGTCATAAGA
<i>Trp53</i> cDNA	GCAGGGTGTCACGCTTCTCCG	GGCTGGTGATGGGGACGGGA
<i>Cdkn2a</i> Ex. 1a (specific for p16)	AGCAGTGTTTTTCAGGGGTGT	CAGATGGGACACTCCTTGCC
<i>Cdkn2a</i> Ex. 1b (specific for p19)	AAGTTAACCGGAGCGAAAGC	CATCTCCCGGGA ACTAAGCC
<i>Cdkn2a</i> Ex. 2	AGGCAAGTGCTGTGTGTCTA	TCAGGCTCATTTGGGTTGCT

<i>Cdkn2a Ex. 3</i>	GAGCCTAGCAAAGGGGGTTG	ATGGGATGGGGAACCAGAGT
<i>p16^{Ink4a} cDNA</i>	AACTCGAGGAGAGCCATCTG	GCCACATGCTAGACACGCTA
<i>p19^{Arf} cDNA</i>	CTTCTCACCTCGCTTGTCAC	AGCTCTGCTCTTGGGATTGG

Experiment 1

p53*

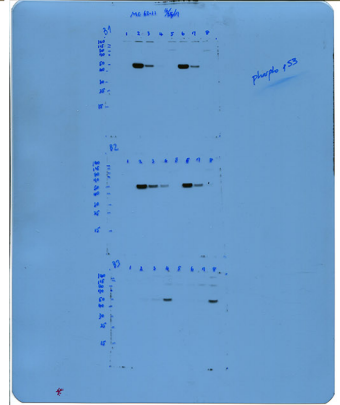
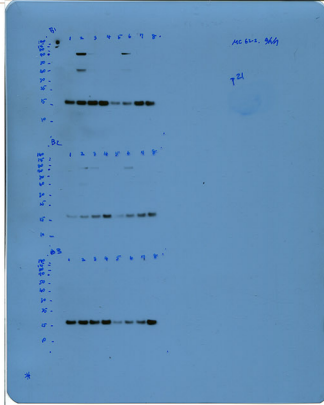
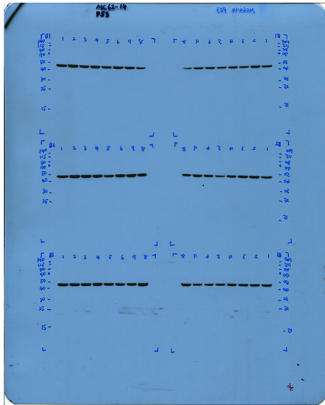
p21

phospho-p53

B1

B2

B3



beta-actin

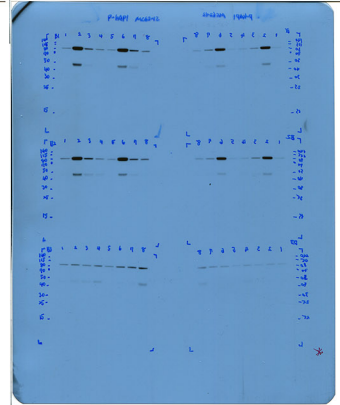
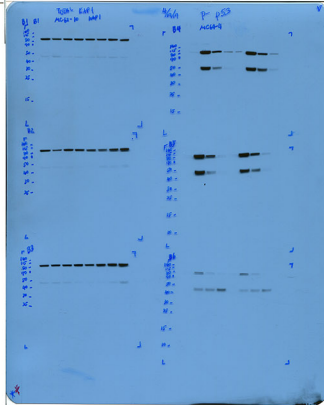
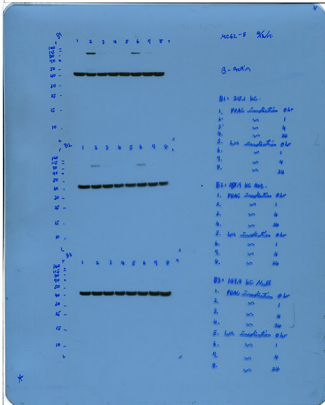
Kap-1 phospho-p53

phospho-Kap1*

B1

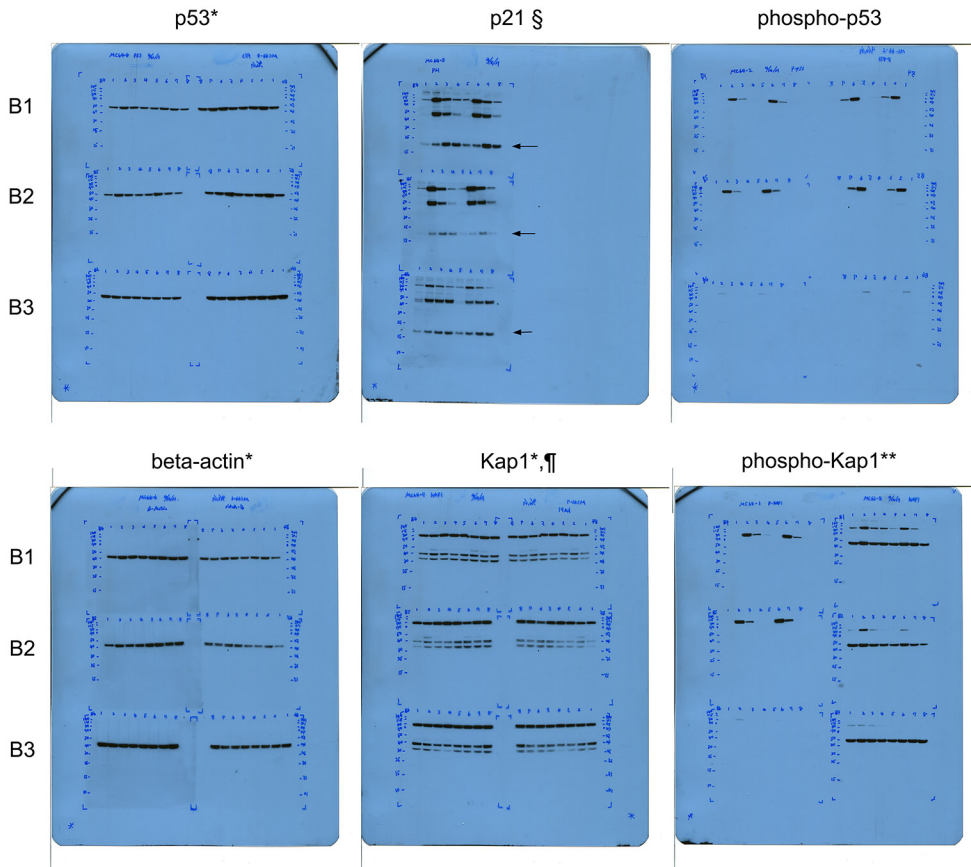
B2

B3



* Two different exposures are shown to the left and right.

Experiment 2



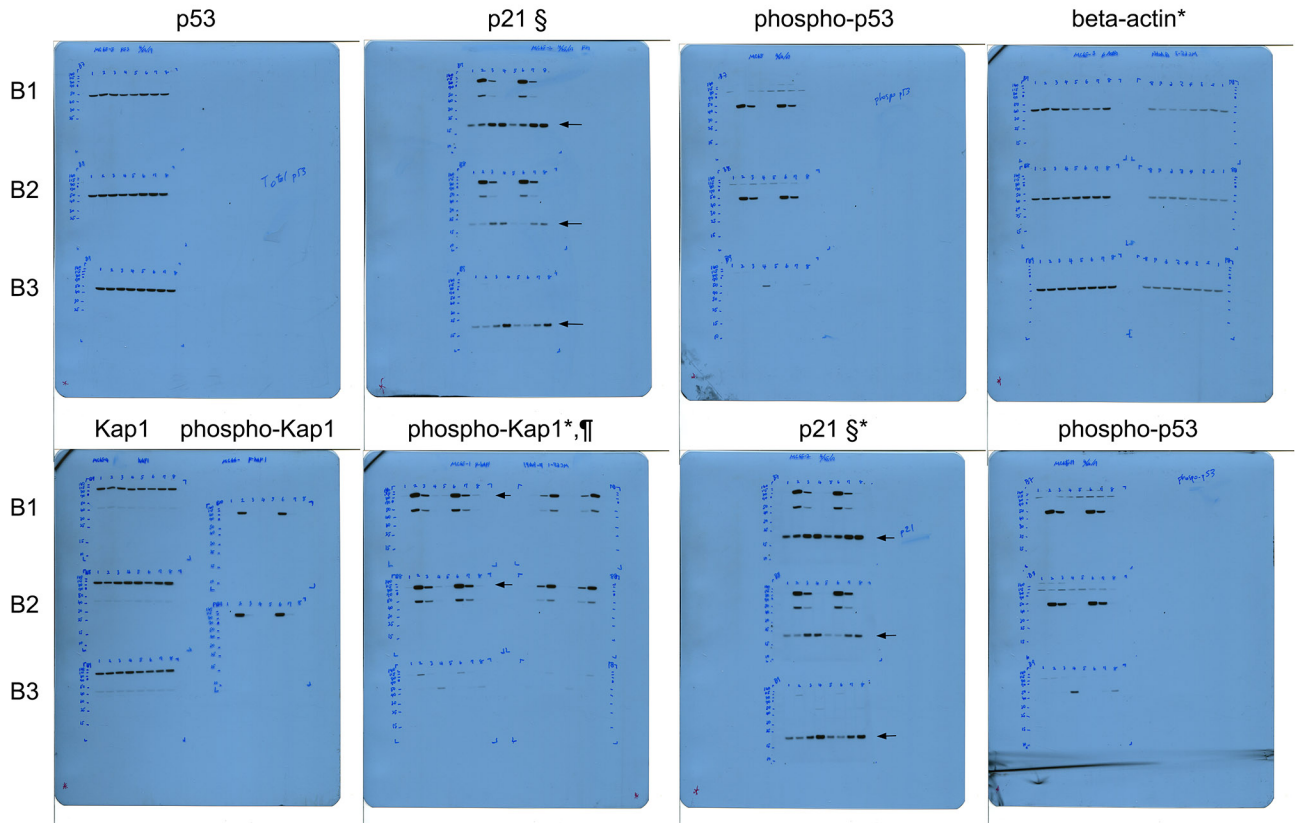
* Two exposures (left and right).

** Two exposures; membrane to the right was stripped twice and hybridized for p53 and Kap1.

§ Membrane was hybridized for p21 (arrow) and then stripped twice and hybridized for p-Kap1 and p-p53.

¶ The 30-40 kd doublet is unspecific.

Experiment 3



* Different exposures (left and right)

§ Membrane was hybridized for p-Kap1 and p-p53 (upper bands), stripped and hybridized for p21 (arrows).

¶ Membrane was hybridized for p-Kap1 (arrows), stripped and hybridized for p-p53

Western Blot samples

B1: KC 215.1

B2: 255.9 KCAD+

B3: 149.4 KCADD

PDAC: primary tumor cells

LM: liver metastasis cells

Cell were irradiated (4 Gy X-ray) and harvested at different time points. This experiment was repeated 3 times.

1. PDAC, 0hr
2. PDAC, 1hr
3. PDAC, 4hr
4. PDAC, 12hr
5. LM, 0hr
6. LM, 1hr
7. LM, 4hr,
8. LM, 24hr